AMENDMENTS TO THE SPECIFICATION

Please enter the following amendments to the specification, without prejudice, without admission, without surrender of subject matter, and without any intention of creating any estoppel as to equivalents:

Please delete the paragraph on page 3, line 15, to page 4, line 6 and replace it with the following paragraph:

The fiber has a well-defined structural organization with each of its three domains, the tail, the shaft, and the knob, performing a number of functions vital for the virus. The short amino terminal tail domain (46 amino acid residues in Ad2 and Ad5 fibers) of the fiber protein is highly conserved among most adenoviral serotypes. In addition to being involved in the association with the penton base protein through an FNPVYD **(SEQ ID NO: 15)** motif at residues 11-16, which results in anchoring the fiber to the adenoviral capsid, the tail domain also contains near its amino terminus the nuclear localization signal KRλR (where λ indicates a small amino acid residue), which directs the intracellular trafficking of newly synthesized fibers to the cell nucleus, where the assembly of the adenoviral particle takes place.

Please delete the paragraph on page 12, line 5, to page 13, line 5 and replace it with the following paragraph:

Attempts to generate adenoviral vectors possessing expanded tropism involved incorporation of short peptide ligands into either the carboxy terminal or so-called HI loop of the knob of the Ad fiber protein. Although these studies demonstrated the feasibility of genetic targeting of Ad and showed the potential utility of such vectors in the context of several disease models (7, 8), further progress in this direction has been hampered by the structural conflicts often observed as a result of modification of the fiber structure. Due to the rather complex structure of the fiber knob domain, even minor modifications to this portion of the molecule may destabilize the fiber, thereby rendering it incapable of trimerization and, hence, non-functional. The upper size limit for a targeting ligand to be incorporated into Ad5 fiber is about 30 amino acid residues (5, 9), which dramatically narrows the repertoire of targeting moieties, thereby limiting the choice of potential ligands and, therefore, cell targets. The task of adenoviral targeting is further complicated by the need to ablate the native receptor-binding sites within the fiber of an adenoviral vector to make it truly targeted. As a result of these limitations, only a handful of heterologous peptide ligands (oligo lysine, FLAG, RGD-4C **(SEQ ID NO: 14)**,

RGS(His)$_6$ **(SEQ ID NO: 16)**, and HA epitope) have been successfully used in the context of Ad5 fiber modification during last several years.

Please delete the paragraph on page 16, lines 5-11 and replace it with the following paragraph:

Figure 1 shows the generation of Ad5 fiber-T4 fibritin chimera containing targeting ligand. Figure 1A shows the schema showing key components of the fiber-fibritin-ligand chimera and their sources. The tail of the fiber anchors the fiber-fibritin-6His chimera **(SEQ ID NO: 13)** in the Ad virion; a fragment of the fibritin protein provides trimerization of the molecule; while the 6His **(SEQ ID NO: 17)** ligand mediates binding to an artificial receptor.

Please delete the paragraph on page 16, line 19, to page 17, line 19 and replace it with the following paragraph:

Figure 2 shows the structure of the fiber-fibritin-6H protein chimera **(SEQ ID NO: 13)**. The FF/6H gene assembled by overlap extension PCR encodes a 373 amino acid long protein chimera which consists of the amino terminal segment of Ad5 fiber protein genetically fused with the carboxy terminal portion of the T4 fibritin protein, followed with the linker and the 6His-containing **(SEQ ID NO: 17)** ligand. The beginning of the third pseudorepeat of the fiber shaft domain (GNTLSQNV) (SEQ ID NO. 11) is joined to the fibritin sequence starting with the fragment of the insertion loop (SQN) preceding the sixth coiled coil segment of the α-helical central domain of the fibritin (VYSRLNEIDTKQTTVESDISAIKTSI) (SEQ ID NO. 12). The sequence SQNV **(SEQ ID NO: 18)** present in the native structures of both fusion partners was chosen as the hinge between the two molecules in order to minimize potential structural conflicts between the β-spiral configuration of the fiber shaft and the triple α-helix of the central domain of the fibritin. The segments of the fibritin sequence localized between every two adjacent coiled coils are the insertion loops which provide some degree of flexibility needed for optimal ligand presentation. A peptide linker is incorporated between the carboxy terminal trimerization domain (foldon) of the fibritin and the six histidine containing ligand to extend the ligand away from the carrier protein in order to facilitate binding to the target receptor.

Please delete the paragraph on page 18, lines 1-10 and replace it with the following paragraph:

Figure 3 shows the Ad-mediated gene transfer to 293/6H cells. 293/6H cells were derived by transfection of 293 cells with a recombinant plasmid expressing an artificial receptor (AR),

which consists of an anti-5His **(SEQ ID NO: 19)** scFv genetically fused with the transmembrane domain of the PDGF receptor. Due to the presence of both CAR and AR on the surface of these cells, 293/6H are susceptible to infection by both the Ad with the wild type fibers and the Ad incorporating the FF/6H chimera. Importantly, each virus i s capable of binding to only one type of receptor, CAR or AR. The progenitor cell line, 293, is refractory to Ad5LucFF/6H infection.

Please delete the paragraph on page 19, lines 10-16 and replace it with the following paragraph:

Figure 5B shows Western blot analysis of FF/6H chimeras incorporated into Ad5LucFF/6H virions. Proteins of denatured Ad5LucFF/6H virions, lane 2, were separated on a 10% SDS-PAGE gel and then probed with anti-Ad fiber tail mAb 4D2, anti 5His **(SEQ ID NO: 19)** mAb Penta-His and anti-fibritin mouse polyclonal antibodies. Wild type Ad5, lane 1, and Ad5LucFc6H, a virus containing fibers with carboxy terminal 6His tags **(SEQ ID NO: 17)**, lane 3, were used as controls.

Please delete the paragraph on page 19, line 17, to page 20, line 18 and replace it with the following paragraph:

Figure 6 shows the binding of Ad5LucFF/6H virions to Ni-NTA-agarose. Wild type Ad5 or Ad5LucFF/6H were incubated with an aliquot of Ni-NTA-resin for one hour. The matrix was pelleted by centrifugation and the supernatant was removed and then incubated with a second aliquot of Ni-NTA-agarose. Aliquots of material subsequently eluted from the resin, as well as an aliquot of the material present in the supernatant after two sequential incubations with the resin, were separated on a 10% SDS-PAGE gel and then stained (Figure 6A) or probed with either anti-fiber tail mAb 4D2 (Figure 6B) or with anti-5His **(SEQ ID NO: 19)** mAb Penta-His (Figure 6C). Lane 1, aliquot of the virus prior to incubation with Ni-NTA-agarose; lane 2, material bound to the first aliquot of the resin; lane 3, material bound to the second aliquot of the resin; lane 4, material remaining in the supernatant after two sequential bindings to the resin. Incomplete binding of Ad5LucFF/6H virions to Ni-NTA agarose is most likely due to the small size of pores in the Sepharose CL-6B used as the matrix for manufacturing Ni-NTA-agarose. According to the manufacturer's specifications, the size of those pores does not allow protein molecules with molecular mass larger that 4MDa to enter the pores. Thus, the Ni-NTA groups which are localized on the surface of the Sepharose particles are accessible to the 6His-tagged

(SEQ ID NO: 17) virions (relatively small percentage), whereas those hidden inside the pores (the majority) are not.

Please delete the paragraph on page 21, line 20, to page 22, line 7 and replace it with the following paragraph:

Figure 8B shows the specificity of Ad5LucFF/6H binding to the artificial receptor. 293/6H cells grown in monolayer culture were pre-incubated with various concentrations of either the truncated form of fibritin or fibritin carrying a carboxy terminal 6His tag **(SEQ ID NO: 17)**, fibritin-6H, prior to infection with Ad5LucFF/6H. Luciferase activities detected in the lysates of infected cells twenty hours postinfection were given as percentages of the activity in the absence of blocking protein. Each data point was set in triplicates and calculated as the mean of three determinations.

Please delete the paragraph on page 22, lines 8-9 and replace it with the following paragraph:

Figure 9 shows the schema of key components of the fiber-fibritin-RGD/6His chimera **(SEQ ID NO: 16)**.

Please delete the paragraph on page 22, lines 14-21 and replace it with the following paragraph:

Figure 11 shows Western blot analysis of FF.RGD/6H chimeras incorporated into Ad5LucFF/6H virions. Proteins of denatured Ad5LucFF.RGD/6H virions, lane 2, were separated on a 10% SDS-PAGE gel and then probed with anti-Ad fiber tail mAb 4D2, anti-5His **(SEQ ID NO: 19)** mAb Penta-His and anti-fibritin mouse polyclonal antibodies. Wild type Ad5, lane 3, and Ad5LucFc6H, a virus containing fibers with carboxy terminal 6His tags **(SEQ ID NO: 17)**, lane 1, were used as controls.

Please delete the paragraph on page 28, lines 3-15 and replace it with the following paragraph:

In order to provide a receptor-binding ligand, a carboxy terminal six-histidine sequence was connected to the fibritin protein of this fiber-fibritin chimera via a short peptide linker (Fig. 2). The purpose of this maneuver was to demonstrate the feasibility of targeting of fibritin-containing Ad vectors to alternative cell-surface receptors by directing the modified vector to an artificial receptor, which is expressed on the surface of 293/6H cells (Fig. 3). The extracellular domain of this artificial receptor (AR) is an anti-5His **(SEQ ID NO: 19)** single chain antibody,

which is genetically fused with the transmembrane domain of the platelet derived growth factor receptor (13). In addition to receptor binding, this 6His **(SEQ ID NO: 17)** sequence was employed to facilitate the detection and purification of the FF/6H chimeras and Ad virions incorporating this protein.

Please delete the paragraph on page 31, line 10, to page 32, line 14 and replace it with the following paragraph:

Construction of the fiber-fibritin-6His **(SEQ ID NO: 13)** (FF/6H) chimera

Generation of the gene encoding the fiber-fibritin-6His chimera was done in several steps. First, a segment of the fibritin gene was PCR-amplified and used to substitute most of the fiber gene sequence encoding the shaft domain. For this, a portion of the T4 fibritin gene encoding the sixth coiled coil through the C-terminal of the protein was amplified with a pair of primers "FF.F" (GGG AAC TTG ACC TCA CAG AAC GTT TAT AGT CGT TTA AAT G) (SEQ ID NO. 1) and "FF.R" (AGG CCA TGG CCA ATT TTT GCC GGC GAT AAA AAG GTA G) (SEQ ID NO. 2). The product of this PCR encodes a segment of an open reading frame (ORF) containing four amino terminal (GLNT) **(SEQ ID NO: 20)** and three carboxy terminal (KIG) codons of the fiber shaft sequence fused to the fibritin sequence. The reverse primer introduces a silent mutation at the 3' end of the fibritin open reading frame resulting in generation of a unique NaeI-site. Also, NcoI-site was incorporated in the "FF.F" in order to fuse the open reading frame of the fiber and the fibritin. The product of the PCR was then cleaved with NcoI and cloned in the fiber shuttle vector pNEB.PK3.6 (22) cut with NaeI and NcoI. As a result of this cloning, an original NaeI-site in the fiber open reading frame was destroyed, therefore NaeI-site a t the end of the fibritin open reading frame remains unique. The plasmid generated was named pNEB.PK.FF_{BB}. This fusion procedure resulted in an open reading frame, in which the fiber and the fibritin sequence were joined via an SQNV peptide **(SEQ ID NO: 18)** hinge, present at the beginning of the 3rd repeat of Ad fiber shaft as well as at the 6th coil coiled segment of the fibritin.

Please delete the paragraph on page 34, lines 4-12 and replace it with the following paragraph:

An RGS $(His)_6$-encoding sequence **(SEQ ID NO: 16)** was fused to the 3' end of the FF_{BB}LL gene by inserting a synthetic oligo duplex made of oligos "RGS6H.T" (GAT CTA GAG GAT CGC ATC ACC ATC ACC ATC ACT AAT) (SEQ ID NO. 7) and "RGS6H.B" (ATT

AGT GAT GGT GAT GGT GAT GCG ATC CTC TA) (SEQ ID NO. 8) into BamHI-SwaI-digested pXK.FF_{BB}LL. The resultant plasmid was designated pXK.FF/6H. This cloning procedure destroyed both the BamHI- and the SwaI-sites. This completed the derivation of the shuttle plasmid containing the FF/6H gene.

Please delete the paragraph on page 35, line 11, to page 36, line 4 and replace it with the following paragraph:

Characterization of recombinant adenovirus expressing the fiberfibritin-6His **(SEQ ID NO: 13)**(FF/6H) chimera.

For the purposes of preliminary characterization, the FF/6H chimeric protein was initially expressed in *E.coli* and purified on a Ni-NTA-agarose column. Subsequent SDS-PAGE analysis of the purified chimeric protein proved that it is trimeric and that the FF/6H trimers are as stable in an SDS-containing gel as the trimers of the wild type Ad5 fiber (Fig. I B). Efficient binding of the FF/6H protein to a Ni-NTA-containing matrix proved that the 6His ligand **(SEQ ID NO: 17)** was available for binding in the context of this trimeric molecule. According to this analysis, truncated T4 fibritin incorporated into the FF/6H protein was able to direct trimerization of the chimera and also successfully served the purposes of ligand presentation, thereby satisfying two key functional criteria of an ideal fiber-replacing molecule.

Please delete the paragraph on page 37, line 6, to page 38, line 4 and replace it with the following paragraph:

The next goal was to demonstrate that the FF/6H chimeras had been incorporated into the Ad5LucFF/6H capsids. Since fiberless Ad5 virions have been successfully purified on CsCl gradients by others (15, 16), it was possible that the putative Ad5LucFF/6H virions isolated in our study could have lacked FF/6H proteins. This was ruled out by SDS-PAGE of purified Ad5LucFF/6H virions and a Western blot analysis utilizing anti-sera specific to all three major components of FF/6H chimera, the fiber tail, the fibritin and the 6His ligand **(SEQ ID NO: 17)** (Fig. 5A and B). These assays showed that the capsid of Ad5LucFF/6H virions consists of completely matured A d proteins and incorporates full-size FF/6H chimeras. As expected, no wild type fibers were found in this preparation of Ad5LucFF/6H. These findings were further corroborated in an experiment involving binding of purified Ad5LucFF/6H virions to Ni-NTA-resin: in contrast to the Ad vector containing wild type fibers, which did not bind to the matrix, Ad5LucFF/6H demonstrated 6His-mediated **(SEQ ID NO: 17)** binding to the resin (Fig. 6).

Therefore, in addition to its ability to assume a trimeric configuration and bind to a receptor-mimicking molecule, the FF/6H chimera also retained the capacity of being incorporated into mature Ad capsids.

Please delete the paragraph on page 38, line 13, to page 39, line 5 and replace it with the following paragraph:

The ability of Ad5LucFF/6H to deliver a transgene to the target cells was then evaluated in a series of studies employing this viral vector for infection of 293/6H cells expressing an artificial receptor capable of binding proteins and Ad virions possessing a 6His tag **(SEQ ID NO: 17)** (Fig. 3). First, the gene transfer capacity of Ad5LucFF/6H was compared to that of an isogenic Ad vector, Ad5Lucl, bearing wild type fibers (Fig. 8A). The doses of both viruses used in this experiment were normalized based on the particle titers of the viral preparations, which also correlated well with the total protein concentration of the samples. Due to the significant differences in the dissociation constants (k_d) previously determined for the Ad5 fiber/CAR interaction (17), 4x 10^{-9} M, and for the 5 His/anti-5His **(SEQ ID NO: 19)** mA b 3D5 interaction (18), 4.75x10^{-7} M, lower efficiency of the gene transfer for Ad5LucFF/6H vector was expected.

Please delete the paragraph on page 40, lines 1-10 and replace it with the following paragraph:

The next gene transfer experiment employed two different forms of recombinant fibritin proteins as blocking agents, of which only one, fibritin-6H, contained a carboxy terminal 6His tag **(SEQ ID NO: 17)** (Fig. 8B). The purpose of this assay was to provide additional evidence that the backbone of the fibritin molecule does not contribute to binding to AR or any other cell surface receptor. Dose-dependent inhibition of Ad5LucFF/6H infection of 293/6H cells with fibritin-6H, but not with the fibritin lacking the 6His tag **(SEQ ID NO: 17)**, further proved that this tag is the component of the virion solely responsible for the binding of the virus to the AR.

Please delete the paragraph on page 42, line 16, to page 43, line 2 and replace it with the following paragraph:

Characterization of recombinant adenovirus expressing the fiberfibritin-RGD-6His **(SEQ ID NO: 13)** (FF.RGD/6H) chimera

A second adenoviral vector, Ad5luc.FF.RGD/6H, containing fiber-fibritin chimeras incorporating at their carboxy termini two peptide ligands RGD-4C (CDCRGDCFC) (SEQ ID

NO. 14) and 6His **(SEQ ID NO: 17)** was generated (Fig. 9). The virus was propagated in 293 cells and purified on CsCl gradient according to standard technique.

Please delete the paragraph on page 43, lines 12-17 and replace it with the following paragraph:

FF.RGD/6H chimeras present in the preparation of Ad5luc.FF.RGD/6H were further identified by Western blot analysis utilizing a set of antibodies specific to each of the component of the chimeric protein. The presence of the fiber tail domain, the fibritin fragment and the 6His tag **(SEQ ID NO: 17)** was confirmed by using relevant mono- and polyclonal antibodies (Fig. 11).

Please delete the paragraph on page 43, line 18, to page 44, line 7 and replace it with the following paragraph:

Association of the FF.RGD/6H chimeras with the Ad5luc.FF.RGD/6H particles was proved by incubating purified Ad5luc.FF.RGD/6H virions with Ni-NTA-sepharose which is designed for purification of the 6His-tagged **(SEQ ID NO: 17)** proteins. In contrast to control adenoviral vector containing wild type fibers which did not bind to Ni-NTA, Ad5luc.FF.RGD/6H was efficiently retained on the column. The presence of all major adenoviral capsid proteins in the material eluted from the resin with imidazole suggested that the Ad5luc.FF.RGD/6H virions were anchored to Ni-NTA-sepharose by virtue of the 6His-containing **(SEQ ID NO: 17)** fiber-fibritin chimeras associated with the virions (Fig. 12).

Please delete the paragraph on page 44, line 18, to page 45, line 6 and replace it with the following paragraph:

To evaluate the gene transfer capacity of Ad5luc.FF.RGD/6H, the virus was employed for gene delivery experiments utilizing two different cell lines: 293 and 293/6H. The latter of the two lines is the derivative of 293 cells constitutively expressing artificial receptor capable of binding 6His-tagged **(SEQ ID NO: 17)** proteins. The luciferase-expressing adenoviral vector isogenic to Ad5luc.FF.RGD/6H but incorporating the wild type fibers was used in these experiments as a control. The gene transfer with the control virus was done at one multiplicity of infection (MOI), whereas Ad5luc.FF.RGD/6H was used at different MOIs.

Please delete the paragraph on page 45, lines 7-12 and replace it with the following paragraph:

As shown in Figure 14, Ad5luc.FF.RGD/6H can deliver a luciferase reporter to both types of cells, although with rather different efficiencies (luciferase expression in naive 293 cells was always lower than in 293/6H cells), thereby suggesting that both the RGD-4C **(SEQ ID NO: 14)** and the 6His peptides **(SEQ ID NO: 17)** incorporated within the FF.RGD/6H chimeras functioned as targeting ligands.

After the last page of the specification (page 47), and before the first page of claims, kindly replace the previously filed Sequence Listing with the enclosed pages entitled --Sequence Listing--.